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Stability Indicating Method Development and Validation for the Simultaneous Estimation of Encorafenib and Binimetinib In Bulk and Pharmaceutical Dosage Form by Uplc

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Abstract

The objective of the present investigation has been to develop a simple, Accurate, precise method for the simultaneous estimation of the Encorafenib and Binimetinib and its application to its pharmaceutical dosage forms. Chromatogram was run through HSS C18 100 x 2.1 mm, 2µm. Mobile phase containing 0.01N Kh2po4: Acetonitrile taken in the ratio 70:30 was pumped through column at a flow rate of 0.3 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 248 nm. Retention time of ERN and BMN were found to be 1.182 min and 1.804. %RSD of the ERN and BMN were and found to be 0.6 and 0.6 respectively. %Recovery was obtained as 99.72% and 99.77% for ERN and BMN respectively. LOD, LOQ values obtained from regression equations of ERN and BMN were 0.24, 0.74 and 0.03, 0.08 respectively. Regression equation of ERN is y = 26691x + 7831.5 and y = 54983x + 1850.4 of BMN. Retention times were decreased and that run time was decreased. The validation of method was carried out utilizing ICH-guideliness.so this newly developed method for the estimation of ERN and BMN was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio equivalence studies and in clinical pharmacokinetics studies in near future. The method developed was simple and economical that can be adopted in regular Quality control test in Industries

Keywords: RP-UPLC, Encorafenib (ERN) and Binimetinib (BMN), Method development,ICH Guidelines.

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INTRODUCTION

ERN Chemical name is methy l N-[(2S)-1-[[4-[3-[5-chloro-2-fluoro-3-(methanesulfonamido) phenyl]-1-propan-2-ylpyrazol-4-yl]pyrimidin-2-yl]amino]propan-2-yl]carbamate. BMN Chemical name is 6-(4-bromo-2-fluoroanilino)-7-fluoro-N-(2-hydroxyethoxy)-3-methylbenzimidazole-5-carboxamide. ERN and BMN are a combination therapy that blocks the activity of different molecules within cancer cells that cause the cancer to grow and spread. This combination of targeted therapies treats advanced melanoma more effectively than the single drugs while producing less serious side effects. In 2018, the U.S. Food and Drug Administration (FDA) approved the use of encorafenib in combination with binimetinib to treat patients with unresectable or metastatic melanoma with a $BRAF^{V600E}$ or $BRAF^{V600K}$ mutation. [1-7]

In reported RP-HPLC[8] method the separation was done by using Agilent C18 column with mobile phase of 0.1M dipotassium hydrogen phosphate (pH 4.0) and methanol in 50:50 vol/vol ratio. The Binimetinib was eluted at 3.448 min and encorafenib at 5.795 min. Linearity ranges are 7.5-22.5 µg/ml and 37.5-112.50 µg/ml with regression coefficient values of 0.9996 and 0.9997 for BMN and ERN respectively. In reported LC–MS/MS Assay method[9] Chromatographic separation of ERN and BMN and avitinib (an internal standard) was achieved using an isocratic mobile phase on a Hypersil BDS C18 column. The linear range for ERN and BMN in the human liver microsome (HLM) matrix was found to be 5–500 ng/mL (R 2 ≥ 0.999). In reported UP-HPLC [10] method the separation was done by using an HSS C18 (100 × 2.1 mm, 1.8 m) reverse phase column, and the mobile phase composition of 0.01N KH2PO4 buffer (pH3.5) and acetonitrile in the proportion of 55:45 was processed through a column at a flow rate of 1 mL/min. The temperature of the column oven was kept at 30°C, and the wavelength maximum of detection system was set to 294 nm.

Literature reviewdiscloses that very few different methods were reported for the analysis of ERN and BMN in bulk and formulations by RP-HPLC[8], LC–MS/MS [9] and UP-HPLC [10] After detailed studies, in present studies plan to develop a new, sensitive, economical method for its analysis in bulk and formulation and validated as per ICH norms.[11-13]

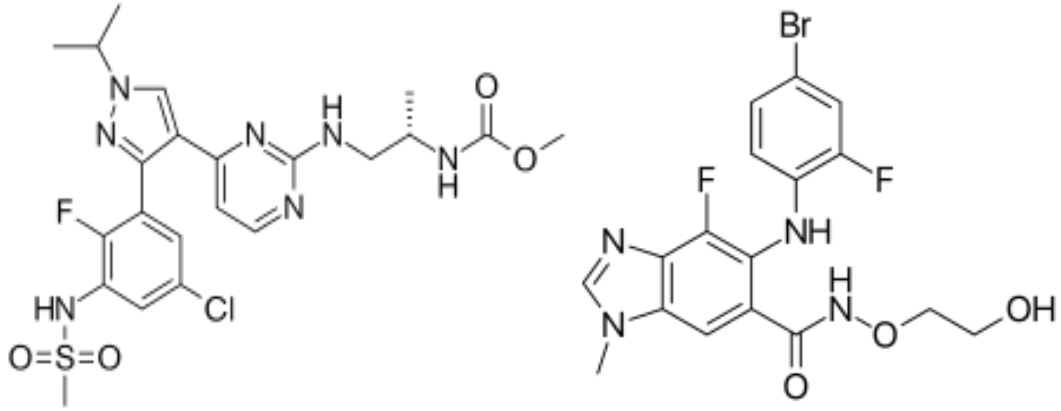


Figure 1. Structure of ERN and BMN

1. MATERIALS AND METHODS

1.1. Materials

ERN and BMN pure drugs (API), Combination ERN and BMN Tablet formulation (BRAFTOVI), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All chemicals, HPLC grade, Merck, are purchased from local distributor.

1.2. Instruments

UPLC instrument used was of WATERS ACQUITY SYSTEM UPLC 2965 with Auto Injector and Acquity TUV detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of ERN and BMN. Sonicator (Ultrasonicator-BVK enterprises)**,** P^H meter (Thermo scientific)**,** Micro balance (Sartorius)**,** Vacuum filter pump (Welch) are the other instruments used for this study.

1.3. Analytical methodology

1.3.1. Preparation of buffer(0.01N KH_2PO_4 Buffer)

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

1.3.2. Standard/ Working solution preparation

Preparation of Standard stock solutions: Accurately Weighed and transferred 22.5mg of ERN, and 2.25mg of BMN working Standards into 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (450µg/ml ERN, and 45µg/ml of BMN)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (45µg/ml ERN, and 4.5µg/ml of BMN)

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (4500µg/ml ERN, and 450µg/ml of BMN).

Preparation of Sample working solutions (100% solution): 0.1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(45µg/ml ERN, and 4.5 µg/ml of BMN).

Diluent: Based up on the solubility of the drugs, diluent was selected, Water and Acetonitrile taken in the ratio of 50:50.

1.3.3. Linearity

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of ERN and BMN are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 11.25ppm, 22.5ppm, 33.75ppm, 45ppm, 56.25ppm, 67.5ppm of ERN and 1.125ppm, 2.25ppm, 3.375ppm, 4.5ppm, 5.625ppm, 6.75ppm of BMN respectively.

1.3.4. Precision

Accurately Weighed and transferred 22.5mg of ERN, and 2.25mg of BMN working Standards into 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. ((450µg/ml ERN, and 45µg/ml of BMN). 0.1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (45µg/ml ERN, and 4.5µg/ml of BMN).

1.3.5. Accuracy

Accurately Weighed and transferred 22.5mg of ERN, and 2.25mg of BMN working Standards into 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (450µg/ml ERN, and 45µg/ml of BMN). From this solution 0.5, 1.0 and 1.5ml was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent to produce 50, 100, 150% of spiked solution respectively.

1.4. Validation Procedure [24]

The analytical method was validated as per ICH Q2(R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ) and forced degradation.

1.4.1. System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

1.4.2. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and the amount of the drug present, percentage of recovery and related standard deviation were calculated.

1.4.3. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the present method was assessed in terms of repeatability, intra-day and inter-day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

1.4.4. Linearity and range

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample within a given range. The six series of standard solutions were injected for assessing linearity range. The calibration curve was plotted using peak area with concentration of the standard solution and the regression equations were calculated.

1.4.5. LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were separately determined based on the calibration curve. The LOD and LOQ of ABN determined by injecting progressively low concentrations of standard solutions by using the developed method. The LOD and LOQ were calculated as 3.3s/n and 10s/n respectively as per ICH guidelines, where s/n indicates signal-to-noise ratio.

1.4.6. Stress degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q1A (R2).The degradation peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions (acid, alkali, oxidation, thermal, UV, water) to obtain the degradation of about 20%.

1.4.7. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the UPLC system and altered chromatographic conditions such as Flow minus, Flow plus, mobile phase minus, mobile phase plus, temperature minus and temperature plus. The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

2. METHOD DEVELOPMENT

2.1. Optimized method

Trials were performed for the method development by using different column like CHS C18, BEH C18, SB C8, HSS C18, Hibar C18 etc., and the best peak were eluted with Hibar C18 column at 1.182min and 1.804 min respectively with good resolution and Plate count. Optimizedchromatographic conditions were shown in Table 1 and optimizedchromatogram was shown in figure 2.

Table 1. Chromatographic conditions

Mobile phase	Acetonitrile: 0.01N KH_2PO_4 (70:30 v/v)
Flow rate	0.3 ml/min
Column	Hibar C18 (2.1 x 100mm, 2µm)
Detector wavelength	Acquity TUV 248nm
Column temperature	30°C
Injection volume	1.00µL
Run time	3 min
Diluent	Water and Acetonitrile in the ratio 50:50

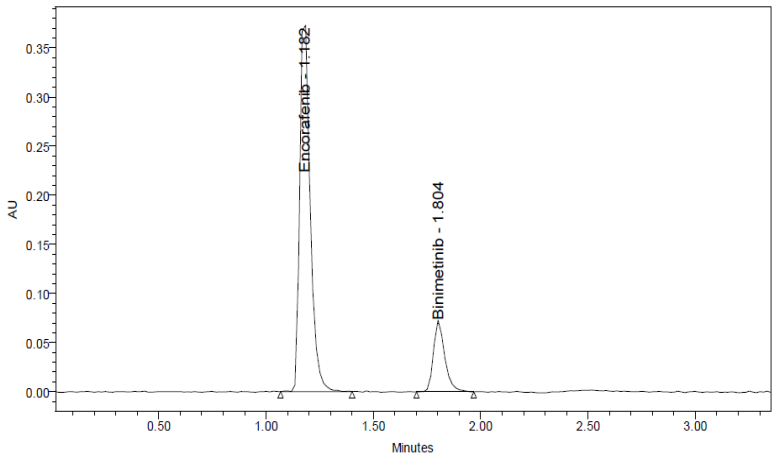


Figure 2. Optimized chromatogram

2.2. System suitability

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. System suitability parameters were shown in table 2 and chromatogram was shown in figure 3.

Table 2. System suitability parameters of ERN and BMN

S. No.	ERN			BMN			
Injection	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	1.184	2737	1.28	1.785	5242	1.30	6.3
2	1.184	2733	1.27	1.818	5205	1.31	6.3
3	1.183	2809	1.29	1.822	5455	1.31	6.4
4	1.186	2835	1.27	1.824	5275	1.30	6.4
5	1.183	2845	1.27	1.805	5422	1.32	6.5
6	1.184	2948	1.29	1.743	5284	1.31	6.5

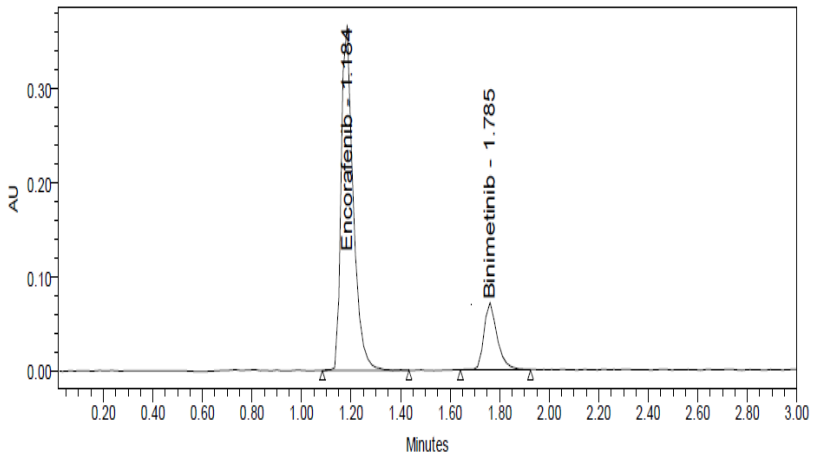


Figure 3. System suitability Chromatogram

3. METHODS FOR VALIDATION

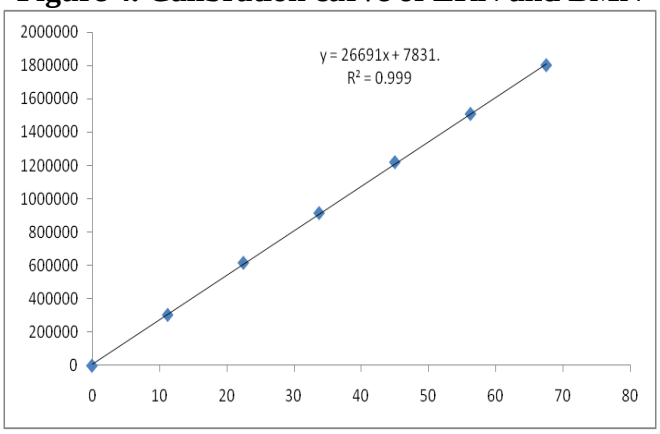
3.1. Linearity

To demonstrate the linearity of assay method, Six linear concentrations of ERN (11.25-67.5µg/ml) and BMN (1.125-6.75µg/ml) were injected in a duplicatemanner. Average areas were mentioned above and linearity equations obtained for ERN was y = 26691x + 7831.5 and of BMN was y = 54983x + 1850.4.Correlation coefficient obtained was 0.999 for the two drugs.

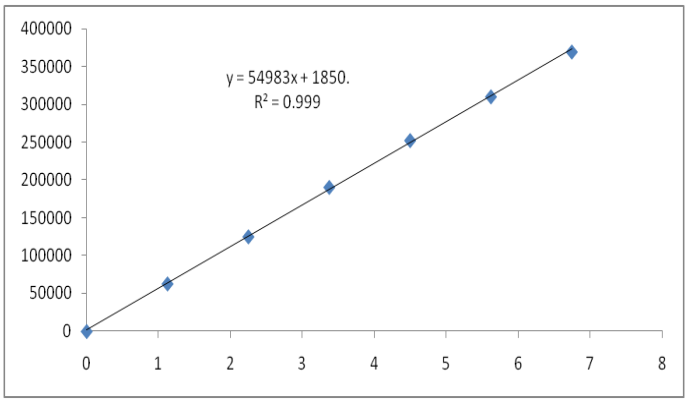
Table 3. Linearity table of ERN and BMN

ERN		BMN	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
11.25	304516	1.125	62963
22.5	616145	2.25	125276
33.75	914015	3.375	190684
45	1218545	4.5	252658
56.25	1507733	5.625	310523
67.5	1799605	6.75	369820

Figure 4. Calibration curve of ERN and BMN



Calibration curve of ERN



Calibration curve of BMN

3.2. Precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.6% and 0.6% respectively for Encorafenib and Binimetinib.As the limit of Precision was less than "2" the system precision was passed in this method. System precision values were shown in Table 4.

Table 4. System precision table of ERN and BMN

S. No	ERN		BMN	
	Peak area	Day-Day precision Peak area	Peak area	Day-Day precision Peak area
1.	1196331	1187190	250116	248919
2.	1203748	1173581	250518	252524
3.	1207532	1177991	248185	253975
4.	1206807	1178843	252345	250114
5.	1194286	1204569	250743	248356
6.	1189969	1192273	248809	251122
Mean	1200468	1185741	250119	250835
S.D	7260.2	11443.5	1480.5	2150.9
%RSD	0.6	1.0	0.6	0.6

3.3. Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.72% and 99.77% for ERN and BMN respectively. Recovery studyvalues were shown in Table 5.

Table 5. Recovery studies of ERN and BMN

% Level	Amount Spiked (µg/mL)		Amount recovered (µg/mL)		% Recovery		Mean %Recovery	
	ERN	BMN	ERN	BMN	ERN	BMN	ERN	BMN
50%	22.5	2.25	22.42844	2.2275	99.68	99.00	99.72%	99.77%
	22.5	2.25	22.38097	2.2109	99.47	98.26		
	22.5	2.25	22.08398	2.2265	98.15	98.95		
100%	45	4.5	44.77646	4.534167	99.50	100.76		
	45	4.5	45.30975	4.473475	100.69	99.41		
	45	4.5	44.88961	4.499538	99.75	99.99		
150%	67.5	6.75	67.48396	6.811434	99.98	100.91		
	67.5	6.75	67.81168	6.792338	100.46	100.63		
	67.5	6.75	67.33118	6.748269	99.75	99.97		

3.4. Robustness

Robustness conditions like Flow minus (0.27ml/min), Flow plus (0.33ml/min), mobile phase minus (75B:25A), mobile phase plus (65B:35A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. Robustness data were shown in table 6.

Table 6. Robustness data of ERN and BMN

S. No.	Condition	%RSD of ERN	%RSD of BMN
1	Flow rate (-) 0.27ml/min	0.5	0.9
2	Flow rate (+) 0.33ml/min	0.6	0.6
3	Mobile phase (-) 60B:40A	1.0	0.3
4	Mobile phase (+) 50B:50A	0.4	0.8
5	Temperature (-) 27°C	0.9	0.9
6	Temperature (+) 37°C	0.5	0.2

3.5. LOD and LOQ

LOD and LOQ were estimated from the signal-to-noise ratio. The LOD of ERN and BMN was found to be 0.18µg/ml and the LOQ was 0.55µg/ml respectively. LOD and LOQ values were shown in table 7. LOD and LOQ Chromatograms were shown in figure 5 & 6 respectively.

Table 7. Sensitivity table of ERN and BMN

Molecule	LOD	LOQ
ERN	0.24	0.74
BMN	0.03	0.08

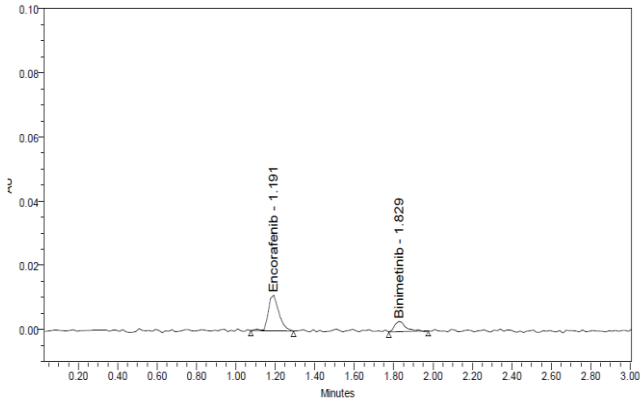


Figure 5. LOD Chromatogram ofERN

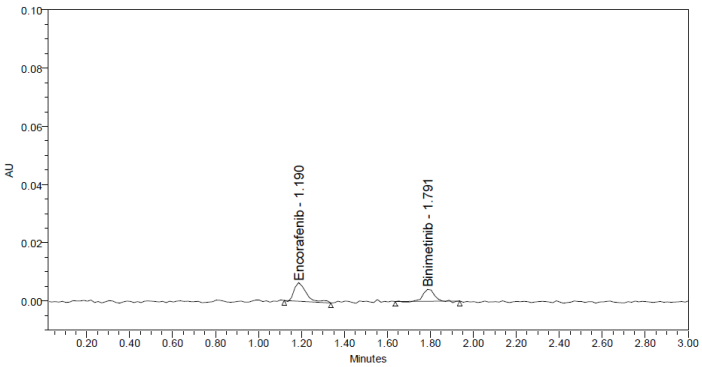


Figure 6. LOQ Chromatogram of ofBMN

3.6. Degradation Data

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Degradation values were shown in table 8.

Table 8. Degradation data of ERN and BMN

Type of degradation	ERN			BMN		
	AREA	% RECOVERED	% DEGRADED	AREA	% RECOVERED	% DEGRADED
Acid	1147244	95.43	4.57	238627	95.21	4.79
Base	1148053	95.50	4.50	239215	95.45	4.55
Peroxide	1159514	96.45	3.55	241755	96.46	3.54
Thermal	1178149	98.00	2.00	244589	97.59	2.41
Uv	1184541	98.53	1.47	246475	98.35	1.65
Water	1193242	99.26	0.74	248252	99.05	0.95

3.7.Assay of marked formulation

BRAFTOVI, bearing the label claim ERN 450mg, BMN 45mg. Assay was performed with the above formulation. Average % Assay for ERN and BMN obtained was 99.91% and 100.14%respectively Assay Data of Marked Formulation were shown in table 9.

Table 9. Assay Data of Marked Formulation of ERN and BMN

S. No.	ERN			BMN		
	Standard Area	Sample area	% Assay	Standard Area	Sample area	% Assay
1	1196331	1204025	100.10	250116	249618	99.60
2	1203748	1216802	101.16	250518	251230	100.24
3	1207532	1199786	99.74	248185	252847	100.89
4	1206807	1195211	99.36	252345	250494	99.95

5	1194286	1204381	100.13	250743	249155	99.42
6	1189969	1190384	98.96	248809	252536	100.76
Avg	1200468	1201765	99.91	250119	250980	100.14
Stdev	7260.2	9104.5	0.757	1480.5	1509.4	0.60
%RSD	0.6	0.8	0.8	0.6	0.6	0.6

4. RESULTS AND DISCUSSION

Literature reviewdiscloses that very few different methods were reported for the analysis of ERN and BMN in bulk and formulations by RP-HPLC[8], LC–MS/MS [9] and UP-HPLC [10] The aim and objectives of the present study was to develop a new UPLC method for rapid, simple and simultaneous quantification, validation and stability studies of ERN and BMN. Trials were performed for the method development by using different column like CHS C18, BEH C18, SB C8, HSS C18, Hibar C18 etc., and the best peak were eluted with Hibar C18 column at 1.182min and 1.804 min respectively with good resolution and Plate count. Optimizedchromatogram was shown in figure 2. The developed method was validated as per ICH guidelines. The validation parameters[13] such as specificity, linearity (R^2 as 0.999), precision (% RSD obtained as 0.6% and 0.6%), accuracy(99.72% and 99.77% for ERN and BMN), robustness and system suitability results were achieved and were within the ICH guidelines [11-13]. The assay of marked Formulation, obtained was 100.14±0.6% is under the limits. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method. Hence it was concluded that the RP-UPLC method developed was very much suit for routine analysis.

5. CONCLUSION

In the present investigation, A simple, accurate, and precise method was developed for the simultaneous estimation of the ERN and BMN in tablet dosage form by the RP-UPLC technique. Retention times of ERN and BMN were found to be 1.182min and 1.804 min respectively. The method was effectively validated in terms of system suitability, linearity, precision, accuracy, range, LOD, LOQ and robustness and stability indicating studies according to ICH guidelines. Hence the developed method can use for estimation of ERN and BMN in quality control departments of pharmaceutical industries and testing laboratories.

6. CONFLICTS OF INTEREST

The authors have no conflict of interest.

7. ACKNOWLEDGEMENT

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